

A simple method for the quantification of diclofenac potassium in oral suspension by high-performance liquid chromatography with UV-detection

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A rapid, simple and low cost method was developed to determine diclofenac potassium (DP) in oral suspension, using a reverse-phase column (C₈, 150 mm x 4.6 mm, 5 µm), mobile phase containing methanol/buffer phosphate (70:30 v/v, pH 2.5), at a flow rate of 1.0 mL/min, isocratic method, and ultraviolet detection at 275 nm. A linear response ($r = 1.0000$) was observed in the range of 10.0-50.0 µg/mL. Validation parameters such as linearity, specificity, precision, accuracy and robustness were evaluated. The method presented precision (repeatability: relative standard deviation = 1.21% and intermediate precision: between-analyst = 0.85%). The specificity of the assay was evaluated by exposure of diclofenac potassium under conditions of stress such as hydrolysis, photolysis, oxidation and high temperature. The method presented accuracy values between 98.28% and 101.95%. The results demonstrate the validity of the proposed method that allows determination of diclofenac potassium in oral suspension and may be used as an alternative method for routine analysis of this product in quality control.

Uniterms: High performance liquid chromatography. Diclofenac potassium. Oral suspension.

Desenvolveu-se método simples, de baixo custo para determinar o diclofenaco potássico (DP) em suspensão oral, usando coluna de fase reversa (C₈, 150 mm x 4,6 mm, 5 µm), fase móvel contendo metanol/tampão fosfato (70:30 v/v, pH 2,5), com fluxo de 1,0 mL/min, método isocrático e detecção no ultravioleta a 275 nm. Observou-se resposta linear ($r = 1,0000$) na faixa de 10,0-50,0 µg/mL. Avaliaram-se parâmetros de validação, como linearidade, especificidade, precisão, exatidão e robustez. O método apresentou precisão (repetibilidade: desvio padrão relativo = 1,21% e precisão intermediária: entre analista = 0,85%). A especificidade do ensaio foi avaliada pela exposição do diclofenaco potássico a condições de estresse, tais como hidrólise, fotólise, oxidação e alta temperatura. O método apresentou valores de exatidão entre 98,28% e 101,95%. Os resultados mostram a validade do método proposto, que permite a determinação de diclofenaco potássico em suspensão oral e pode ser utilizado como alternativa para análise de rotina desse produto no controle de qualidade.

Unitermos: Cromatografia de alta eficiência. Diclofenaco potássico. Suspensão oral.

INTRODUCTION

Diclofenac potassium (DP), 2-[(2,6-dichlorophenyl)-amino]-benzeneacetic acid, is an acid phenyl acetic derivative with anti-inflammatory, analgesic and antithermal properties. It is usually found as potassium and

sodium. This drug is indicated for rheumatoid arthritis, degenerative joint disease, chronic pain associated with cancer and kidney stones and endodontic procedures (Farmacopéia Brasileira, 2010; Sanches, Viletti, 2009; Silva, 2010). Analytical methods have been described in literature for the determination of DP in tablets and human serum by HPLC and spectrophotometry (Elkady, 2010; Naidoo *et al.*, 2009; Souza, Tubino, 2005; Sparidans *et al.*, 2008). Official physicochemical techniques for the quality control of DP in raw material and tablets are

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described in British Pharmacopeia (BP, 2012). The United States Pharmacopeia and Brazilian Pharmacopeia employ techniques such as titration in non-aqueous medium, spectrophotometry and LC (Farmacopéia Brasileira, 2010; USP, 2012).

The development of analytical, safe and reliable methods is a very important tool for the quality control of pharmaceutical products and raw material. For this reason many authors have published articles on the subject (Arend *et al.*, 2009; Nogueira *et al.*, 2007; Rosa *et al.*, 2008).

Due to the lack of a methodology for the determination of DP in oral suspension this study aimed to develop and validate a methodology for the determination of diclofenac potassium in oral suspension for routine analysis in laboratories. This method was validated according to the official guidelines (Brasil, 2003; ICH, 2005; Inmetro, 2007).

MATERIAL AND METHODS

Chemical and Reagents

DP reference substance was obtained from Farmacopeia Brasileira, batch 1053. DP oral suspension (Cataflam[®]) was purchased from the Brazilian market. All chemicals used were of analytical grade and all solvents were LC grade. Methanol and phosphate sodium were purchased from Sigma Aldrich. The purified water used was obtained utilizing the reverse osmosis system (Milli-Q Millipore Corporation[®]).

Equipment and Chromatographic conditions

The development and validation of the assay was performed on a Shimadzu LC system (Kyoto Japan), with an LC-20AT pump, SIL-20A ht automatic injector, CTO-20AC column oven, SPD-M20A photodiode array detector (PDA) and CBM-20A controller with LC solution software. Chromatographic separations were achieved using a Phenomenex[®] Luna C8 (150 x 4.6 mm, 5 μ m) column. The mobile phase and diluent contained a mixture of methanol: buffer phosphate pH 2.5 (70:30 v/v) and methanol: water (70:30 v/v) respectively, flow of 1.0 mL/min, PDA detection at 275 nm. The injection volume was 20 μ L.

Preparation of Standard Solution

10.0 mg of diclofenac potassium reference substance was accurately weighed and dissolved in a 10 mL volumetric flask with diluents. This solution was diluted

appropriately in the range from 10.0 to 50.0 μ g/mL, with an average concentration of 30.0 μ g/mL.

Preparation of Sample Solution

An aliquot equivalent of 2.0 mg of DP, about 1.0 mL of oral suspension was transferred to a 10.0 mL volumetric flask and dissolved with diluents. Then a 1.5 mL aliquot was pipetted into a 10 mL volumetric flask and diluted with the same solvent.

Validation Study

Specificity

Placebo, sample oral suspension and DP reference substance solution were analyzed for the determination of method specificity. The placebo formulation contains: citric acid, sorbic acid, deionized water, strawberry flavor, cellulose microcrystalline, sodium cyclamate, hydroxyethylcellulose, propylparaben, methylparaben, propylene glycol, glyceryl polyoxyethyleno glycol stearate, saccharin sodium.

Moreover, the specificity was evaluated for stress testing (ICH, 2005). The stress conditions follow:

Hydrolytic Conditions

Individually, 25.0 mg of DP reference substance were dissolved in a 25 mL volumetric flask with diluent to generate a concentration of 1 mg/mL. Then 5 mL aliquots were transferred to a 25 mL volumetric flask and dissolved in HCl 0.1 M and NaOH 0.1 M. After 5 and 24 hours, aliquots of 1.5 mL were transferred to a 10 mL volumetric flask and neutralized with NaOH 0.1 M and HCl 0.1 M, respectively.

Oxidative Condition

The equivalent of 25.0 mg of DP reference substance was dissolved in a 25 mL volumetric flask with diluent to generate a concentration of 1 mg/mL. After that, 5 mL aliquots were transferred to a 25 mL volumetric flask and dissolved in hydrogen peroxide 3%. After, 5 and 24 hours, 1.5 mL aliquots were transferred to a 10 mL volumetric flask dissolved in diluents.

Photolytic degradation

The equivalent of 25.0 mg of DP reference substance was dissolved in a 25 mL volumetric flask with diluent to generate a concentration of 1 mg/mL. After that, 5 mL aliquots were transferred to a 25 mL volumetric flask and dissolved in diluents. This solution was exposed to UV light ($\lambda = 254$ nm) for 5 and 24 hours. Then a 1.5 mL

aliquot was transferred to a 10 mL volumetric flask and dissolved in diluents. All the solutions were analyzed in triplicate.

Linearity and range

The linearity was determined by constructing three independent analytical curves, with five concentrations of DP (10.0; 20.0; 30.0; 40.0 and 50.0 µg/mL), which were analyzed in triplicate on three consecutive days. The results were subjected to regression analysis by the least squares method to calculate the calibration curves.

Detection Limit and Quantification Limit

The detection limit (DL) and quantification limit (QL) were based on the standard deviation of the response and the slope of the mean of three calibration curves.

Precision and Accuracy

The precision assay was investigated with respect to repeatability (intra-day) and intermediate precision (inter-day). The repeatability was evaluated by assaying three determinations at concentrations of 10.0; 30.0 and 50.0 µg/mL, during same day and under the same experimental conditions. The intermediate precision was assessed by carrying out the same analysis on 3 different days. The precision was expressed as % of relative standard deviation (RSD).

Accuracy was evaluated from the amount of DP reference substance recovered. The study was performed by adding a known amount of DP standard solution (10.0; 20.0; 30.0 and 40.0 µg/mL) to sample solution. The actual and the measured concentration were then compared. The experiments were repeated three times.

Robustness

The robustness evaluation of the chromatographic method for DP was performed using the method proposed by Youden and Steiner (1975). Five analytical parameters were selected and small variations were induced in the nominal values of the method. The five analytical parameters employed, as well as the variations introduced are shown in Table I. Then, eight runs were performed aiming to determine the influence of each parameter in the final results, according to a matrix of factors in Table II.

The parameters A, a, B, b, C, c, D, d, E, e, were calculated according to equations (equations 1-10) as follows:

According to Nogueira *et al.* (2011), this method will be robust if conditions 1 and 2 are met:

Condition 1: (for factor A and other factors) content of DP – 5% ≤ A ≤ content of DP + 5%.

TABLE I - Analytical parameters and variations to evaluate the robustness of the chromatographic method for DP quantification

Parameter	Variation	
Mobile phase flow rate (mL min ⁻¹)	A - 1.2	a - 0.8
Column supplier	B - Gemini	b - Sunfire
Column temperature (°C)	C - 30	c - 20
Methanol concentration in mobile phase (%)	D - 73	d - 67
Time of ultrasonic bath for preparation sample (min)	E - 25	e - 15

TABLE II - Matrix of factors for the determination of method robustness

Value of factor	Combination assayed							
	1	2	3	4	5	6	7	8
A or a	A	A	A	A	a	a	a	a
B or b	B	B	b	b	B	B	b	B
C or c	C	c	C	c	C	c	C	c
D or d	D	D	d	d	d	d	D	D
E or e	E	e	E	e	e	E	e	E
Results	s	t	u	v	w	x	y	z

Condition 2: (for factor A and other factors) A – a ≤ 3% involving the DP content.

An appropriate amount was transferred into an individual 10 mL volumetric flask, diluted to volume with diluents, and filtered through a 0.45 µm membrane filter (Millipore, Bedford, USA), obtaining the final concentration of 30.0 µg/mL of the active pharmaceutical ingredient. The concentrations of DP presented in samples were determined from the standard curve.

System Suitability

System suitability was evaluated by five replicate analyses of a DP reference substance and sample at a concentration of 30.0 µg/mL. The calculated parameters were: number of theoretical plates, tailing factor and asymmetry (FDA, 1994).

RESULTS AND DISCUSSION

System Suitability

To obtain the best chromatographic method, the mobile phase of methanol and buffer phosphate (70:30 v/v, pH 2.5) was utilized to provide adequate peak and satisfactory results according to criteria evaluated. A

150 mm length octylsilane column was able to obtain a resolution and peak asymmetry and a time analytical short when compared, for example to the method used for the determination of diclofenac in the raw material and tablets.

Specificity

The chromatograms obtained with the specificity test (Figure 1), showed that none of the excipients of the formulation eluted in the same retention time were related to the DP peak. Therefore none of them interfered in its determination.

Furthermore, the interference of potential degradation products was investigated through a forced degradation test. These studies were performed to identify the key factors which will impact the drug stability, as well

as verify that the method is useful for stability studies. Usually the range of degradation is 10% to 30% (Chan *et al.*, 2004).

Basic Conditions

After alkali hydrolysis, the concentration remained constant and no degradation of the DP reference substance was found. The stress test did not show the formation of secondary peaks in the chromatogram.

Acidic Conditions

After zero, 5 and 24- hour hydrolysis times, a reduction in area of around 8.3%, 39.0% and 45.6% occurred respectively, according to Figure 2.

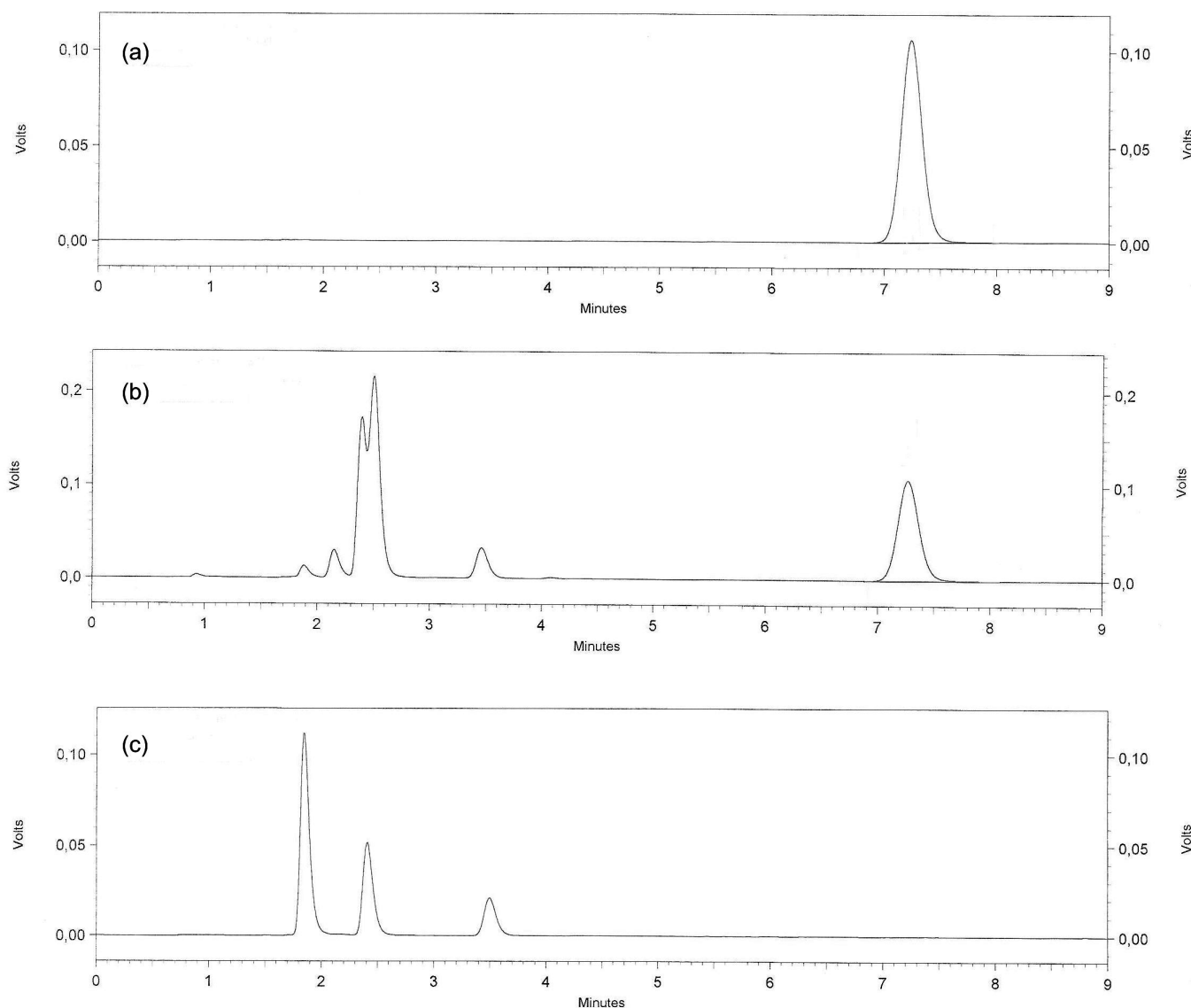


FIGURE 1 - Chromatograms corresponding to: (a) solution of DP reference substance, (b) oral suspension sample and (c) sample placebo.

Oxidative Conditions

After chemical oxidation with H_2O_2 3%, during a 24-hour period, a reduction of area around 14.6% and 43.0% occurred at 5 and 24 hours respectively, according to Figure 3.

Photolytic degradation

Under photolytic condition the average area for DP showed a reduction of about 86.5% at the end of 24 hours, according to Figure 4.

In conclusion, stress degradation tests under the conditions described showed that DP degraded partially under acidic, oxidative and photolytic conditions. Although degradation of the drug occurs under acid and oxidative conditions, there was no appearance

of secondary peaks related to degradation products. Under acidic and oxidative conditions there was no appearance of secondary peaks related to degradation products, which may be explained by the fact that they do not have chromophoric groups in their molecule, present low concentrations or are detected after the end of the run. After 24 hours under photolytic conditions, the drug degraded, as did the appearance of new peaks in the chromatogram related to potential degradation products.

The method proposed can be used to indicate stability because the peak of the parent drug does not suffer interference from other signals in the chromatograms. The purity of peaks was attested with photodiode detector support.

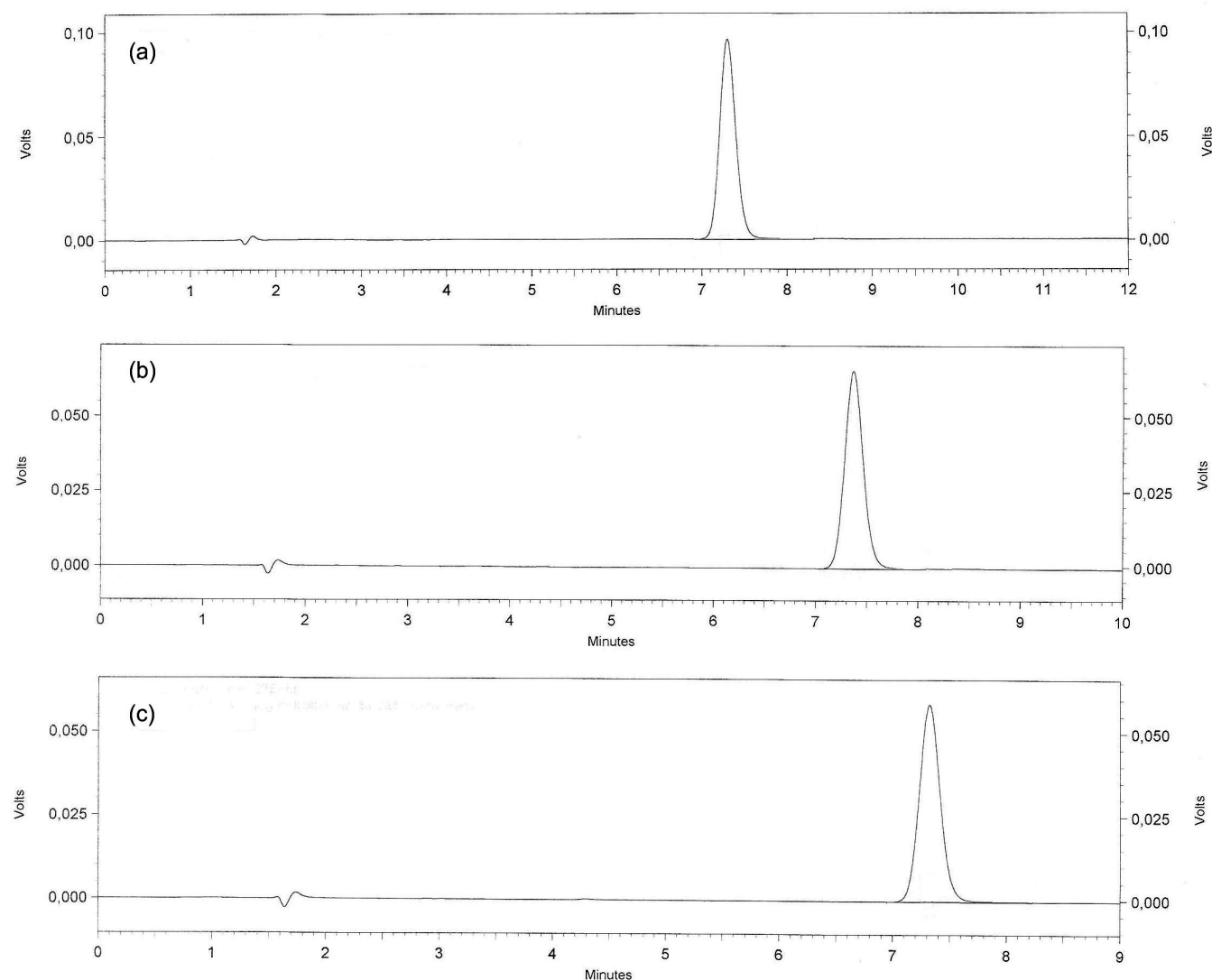


FIGURE 2 - Chromatograms corresponding to: (a) solution of DP reference substance time zero, (b) solution of DP reference substance time 5 hours and (c) solution of DP reference substance time 24 hours after acid hydrolysis conditions.

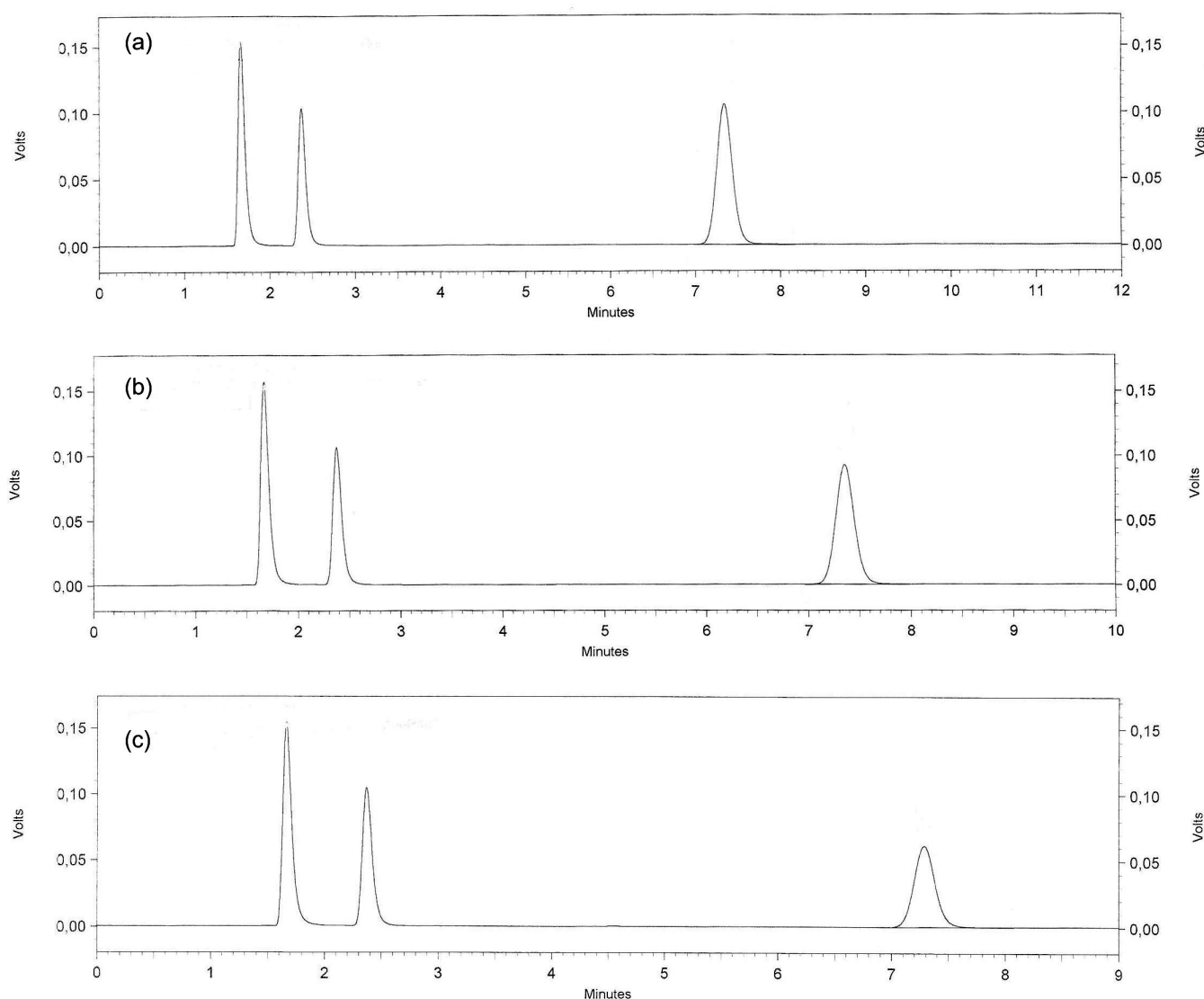


FIGURE 3 - Chromatograms corresponding to: (a) solution of DP reference substance time zero, (b) solution of DP reference substance time 5 hours and (c) solution of DP reference substance time 24 hours after oxidative conditions.

Linearity and range

Linearity was observed over the concentration range of 10.0-50.0 µg/mL, according to Figure 5, with a correlation coefficient of ($r = 1.0000$) according to the criteria established for ($r = 0.9999$) and the linear regression equation $y = 44934x + 36300$ (where, x is concentration and y is the peak absolute area). Assay validity was verified by means of the ANOVA. According to the statistical data, there is a linear relationship between the variables and there is no deviation from linearity ($p < 0.05$). The quantification and detection limits were 0.05 µg/mL and 0.02 µg/mL respectively showing method sensibility.

Precision

The precision of the method was evaluated as

repeatability and intermediate precision and was expressed as RSD%. The mean results were 1.21% and 0.85% of RSD respectively. The limit for this assay is an RSD maximum of 2.0% (USP, 2012).

Accuracy

Accuracy was calculated as the percentage of recovery by the assay of the known added amount DP reference substance in solutions, using three concentration levels and three replicates of each concentration. The average percentage obtained was 98.28%–101.95% satisfying the acceptance criteria for the study.

Robustness

The method described by Youden and Steiner (1975), makes it possible not only to evaluate method

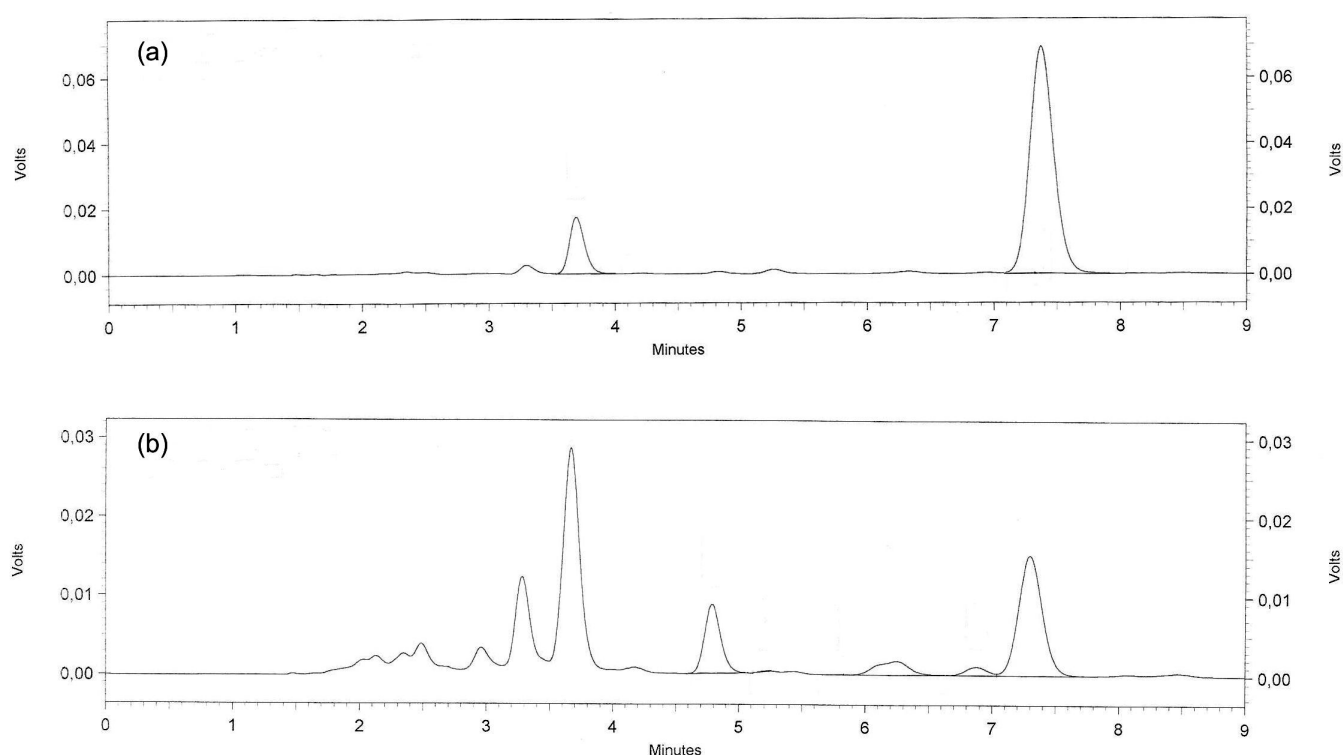


FIGURE 4 - Chromatograms corresponding to: (a) solution of DP reference substance time 5 hours and (b) solution of DP reference substance time 24 hours after photolytic conditions.

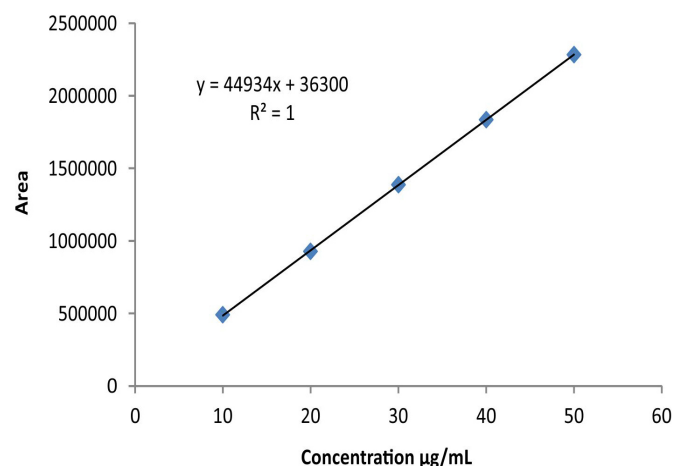


FIGURE 5 - Calibration curve of Diclofenac potassium.

robustness, but also to sort the variation of each parameter in the final results (INMETRO, 2007). Results presented in Tables III and IV, indicate satisfactory robustness of the method.

After variation of some parameters of the method proposed, these variations were not able to significantly change the DP content in the sample, average levels of DP ranging from 98.43% to 102.34%. The results satisfy conditions 1 and 2, and were therefore considered a robust method for the determination of DP in oral suspension.

CONCLUSION

The proposed liquid chromatography method provides specific, accurate, precise and robust results for

TABLE III - Combinations tested to evaluate the robustness of the analytical method, evaluating the conditions 1

Results	Combination assay							
	s	t	u	v	w	x	y	z
Content (%):	99.52	101.50	99.76	100.43	101.34	101.14	98.43	102.34
Average content (%):	100.56							
RSD (%):	1.26							
Acceptable range for content of DP (%):	$96.14\% \leq 101.20 \leq 106.26\%$							

*average of three determinations; RSD – relative standard deviation

TABLE IV - Combinations tested to evaluate the robustness

Content of DP (%)		Difference (%)	Limits for the difference (%) (3%*101.21%)	
A = 100.30	a = 100.81	A – a = - 0.51	3.04	Robust
B = 100.87	b = 100.24	B – b = 0.63	3.04	Robust
C = 99.76	c = 101.35	C – c = - 1.59	3.04	Robust
D = 100.44	d = 100.66	D – d = - 0.22	3.04	Robust
E = 100.69	e = 100.42	E – e = 0.27	3.04	Robust
Average (%)* (A,a,B,b,C,c,D,d,E,e): 100.55				
SD: 0.43				
RSD (%): 0.43				

*average for three determinations; SD: standard deviation; RSD: relative standard deviation

the quantitative determination of DP in oral suspension. Hence, the method developed represents an alternative in the routine of pharmaceuticals laboratories, and is suitable to determine DP in the quality control routine for the pharmaceutical form.

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